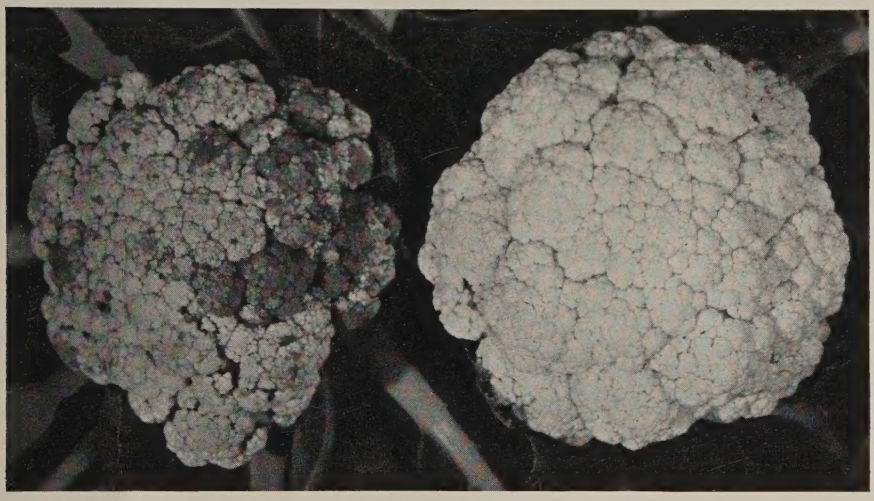
1942.

Bulletin 778 April 1942

29 OCT 1946

Boron Nutrition of Cauliflower in Relation to Browning

C. H. Dearborn



EFFECT OF BORAX ON THE INCIDENCE OF BROWNING OF CAULIFLOWER GROWN IN 12-INCH POTS IN THE GREENHOUSE EXPERIMENT OF 1935

The head on the left, showing surface browning, was grown in Lackawanna silt loam deficient in boron; the normal head on the right was grown in similar soil supplied with 25 pounds of borax to the acre

Published by the
Cornell University Agricultural Experiment Station
Ithaca, New York

Received for publication October 3, 1941

CONTENTS

	PAGE
Description of cauliflower-browning	3
Symptoms of boron deficiency on leaves	4
Materials and methods used in the study	4
Field experiments	4
Soil types and soil treatments	5
Planting and care of the crop	9
Harvesting	9
Greenhouse experiments	9
Laboratory methods	10
Histological technique	10
Microchemical technique	10
Experimental results	11
Field experiments	11
Experiments in 1935	11
Experiments in 1936, 1937, and 1938	12
Greenhouse experiments	13
Experiment 1	13
Experiment 2	15
Histological studies	17
Microchemical studies	22
Discussion and summary	25
References	28

BORON NUTRITION OF CAULIFLOWER IN RELATION TO BROWNING[1]

C. H. Dearborn

During the past twenty years, many cauliflower-growers in the Catskill Mountain area of New York have suffered heavy losses due to a physiological disorder known as "browning". Scattered areas of this browning, or "red rot" as it was commonly known, frequently occurred in the same part of a field every time cauliflower was grown. A survey of approximately 150 farms, covering 443 acres of cauliflower, was made in Delaware County in the fall of 1934 by the assistant county agent and by members of the Department of Vegetable Crops and the Department of Plant Pathology of Cornell University, to ascertain the cause of the disorder. The survey disclosed that no microorganism is associated with the browning, and that the browning is not restricted to any particular soil type, variety, or fertilizer practice. It seemed logical, therefore, to conclude that browning is due either to faulty nutrition (possibly a deficiency of some microelement) or to toxicity.

It was estimated that 30 per cent of the cauliflower crop was unfit for market in 1934 because of the browning of the heads. Growers in the area became so concerned about the losses resulting from browning, that in the winter of 1935 a cauliflower committee of the Delaware County Farm Bureau was chosen to seek aid of the College of Agriculture. In compliance with a request of this cauliflower committee, experiments were started in the field in the spring of 1935 to determine the cause of the browning and means of preventing it.

DESCRIPTION OF CAULIFLOWER-BROWNING

In the cauliflower plant the first symptoms of browning are small spherical areas, water-soaked in appearance, scattered through the pith of the main stem and the lower branches of the head. As the browning progresses these scattered spots increase in size and coalesce. In the more advanced stages the pith areas crack, at first transversely and then vertically, and as a result the stem and branches become hollow. These conditions are illustrated in figure 1 (page 4).

When browning is severe, the surface of several or of all of the branches of the head appears water-soaked for a day or longer, as shown in figure 2. These water-soaked areas soon become rusty-brown and decay rapidly in wet weather, while in a relatively dry atmosphere they become dry, brown, and hard. The affected head is bitter in flavor, both in the raw and in the cooked state, even when no surface browning is evident.

---

[1]Paper no. 245, Department of Vegetable Crops, Cornell University, Ithaca, New York.

Also presented to the Faculty of the Graduate School of Cornell University, September 1939, as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy.

Author's acknowledgments. The writer wishes to express his appreciation particularly to Dr. H. C. Thompson, under whose direction the study was conducted, for suggestions and criticisms given throughout the conduct of the work and for assistance in preparing the material for publication. He is indebted also to Dr. G. J. Raleigh, Dr. O. F. Curtis, and Dr. J. R. Livermore, for advice and suggestions on certain phases of the work. He wishes further to express his appreciation of the cooperation which he received from many cauliflower-growers in Delaware County, to the Delaware County Farm Bureau for financial support, and especially to C. S. Denton, county agricultural agent of Delaware County, for assistance and advice.

SYMPTOMS OF BORON DEFICIENCY ON LEAVES

On cauliflower foliage, boron deficiency is first manifested by a dull green color of all the leaves, followed by fading of the green to a greenish yellow in a band along the apical margin of the older leaves. This color change in the foliage is accompanied by thickening, brittleness, and a downward curling of the older leaves. Simultaneously with these color changes, small blisters arise on the lower side of the petiole where it joins the main stem. Shortly thereafter, blisters form all along the upper side of the midrib, as shown in figure 3. When the leaf elongates, the tissue in the region of the blisters pulls apart, causing the midrib to become checked with small transverse cracks along the upper side. Occasionally there is longitudinal secondary cracking in these blistered regions. Subsequently the edges of the lower leaves develop a distinct purple color, particularly at the terminal of the lateral veins. As the purple edges become continuous around the leaf, the greenish yellow band previously mentioned turns orange-yellow and extends inward from the periphery of the leaf for ½ to 1½ inches, as shown in figure 4. In severe cases the small leaves surrounding the head or the growing point are dwarfed and brittle, and have minute yellow areas with perforations in the center. Death of the small leaves proceeds from the margins at the base of the blade toward the midrib and the apex. Blisters also form at the base and along the midrib of these small leaves.

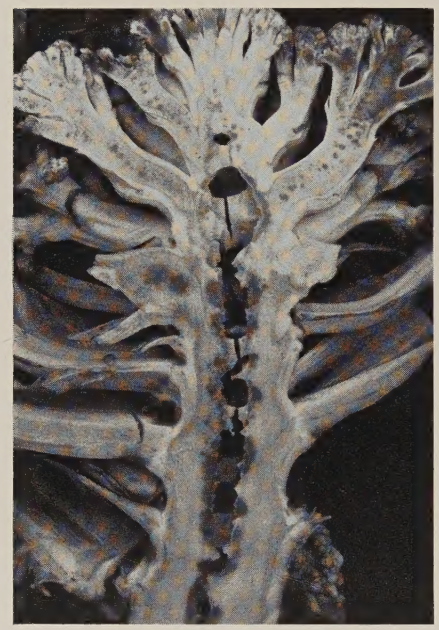


FIGURE 1. LONGITUDINAL SECTION OF A CAULIFLOWER HEAD FROM A PLANT GROWN IN BORON-DEFICIENT SOIL, SHOWING THE HOLLOW STEM AND THE WATER-SOAKED APPEARANCE OF THE BRANCHES OF THE HEAD

MATERIALS AND METHODS USED IN THE STUDY

The experimental work here reported was conducted in field plots in Delaware County, and in soil and nutrient-solution cultures in the greenhouse at Cornell University. The histological and microchemical investigations were made in the laboratories of the Department of Vegetable Crops. The general procedures followed in the field, in the greenhouse, and in the laboratory work are given in this section. Details that will aid in clarifying the results are given under "Experimental Results".

FIELD EXPERIMENTS

The field experiments were conducted in cooperation with cauliflower-growers in the Catskill Mountain area, and with the Delaware County

Farm Bureau, during the growing seasons of 1935, 1936, 1937, and 1938. The experiments of 1935 with six minor elements — magnesium, manganese, iron, copper, zinc, and boron — were conducted to determine whether or not browning resulted from a deficiency of any one of those elements.

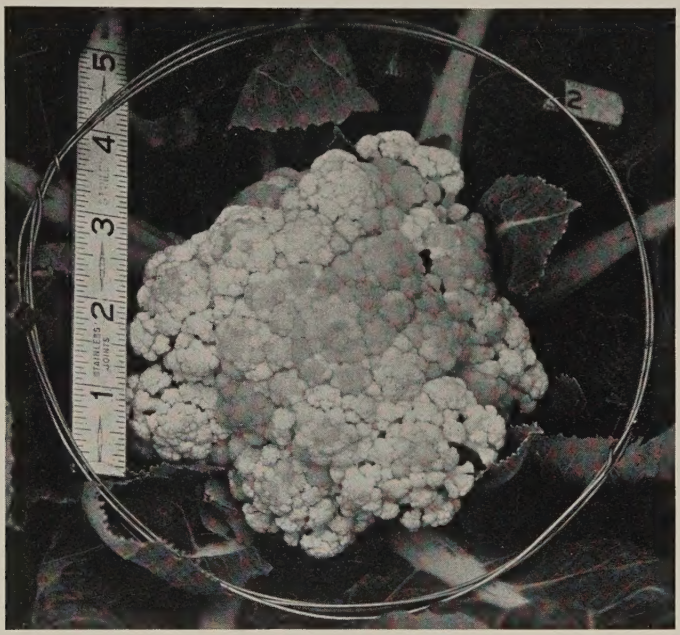


FIGURE 2. EARLY STAGES OF SURFACE BROWNING ON A CAULIFLOWER HEAD PRODUCED ON BORON-DEFICIENT SOIL IN THE GREENHOUSE IN 1935

On some of the branches of the curd the surface appears water-soaked for a day or longer

Since the results indicated clearly that boron deficiency is the cause of the disorder, the experiments in 1936, 1937, and 1938 were conducted to determine the effect of different quantities of boron on plant growth and on the control of browning.

Soil types and soil treatments

All of the principal soil types on which cauliflower is grown in Delaware County were used in this study. The soil types,[2] and the range of elevation above sea level and range of reaction of each, are approximately as follows: Culvers silt loam, 2400 to 3000 feet, pH 4.0 to 4.8; Lackawanna silt loam, 2200 to 2400 feet, pH 4.4 to 5.0; Walton silt loam, 1800 to 2200 feet, pH 4.6 to 5.2; Barbour silt loam, high-bottom phase, 1500 to 1800 feet, pH 4.4 to 5.4.

[2]For further information concerning the soil types in Delaware County, the reader is referred to *Soil Survey of Delaware County, New York*, by Clarence Lounsbury and others (U. S. Dept. Agr., Bur. Chem. and Soils, Ser. 1930, No. 7).

Soils with the reactions mentioned above are not favorable for good growth of the strains of cauliflower available. Therefore an attempt has been made by the growers to decrease the soil acidity to a range of pH 6.0 to 6.7, by using hydrated lime.

FIGURE 3. BORON-DEFICIENCY SYMPTOMS ON THE MIDRIB OF A CAULIFLOWER LEAF

The midrib on the left has blisters all along its upper side; the one in the center shows a large blister with a checked area surrounding it; the one on the right shows the checking effect

For the experiments of 1935, twenty-two fields were selected in which were represented all of the important soil types, varieties of cauliflower, and planting periods. The growers had prepared the soil, had applied broadcast 2 tons of hydrated lime and 2 tons of a 4–8–7 fertilizer to the acre, and had set the plants, before the particular blocks for experimentation were chosen. Areas in two other fields that had not been fertilized were obtained for conducting fertilizer experiments with mixtures of known composition.

The studies to determine the quantity of boron necessary for control of browning consisted of two experiments in 1936, two in 1937, and three in 1938. No two experiments were conducted on the same field during the course of the study. A summary of the general soil treatments, dates of planting, soil reactions, and dates of harvest, is given in table 1.

In the 1935 experiments a wide variety of conditions was encountered, owing to the fact that growers differed in their choice of the various brands of lime and fertilizer which were available. For the most part, 2 tons each of hydrated lime and 4–8–7 fertilizer to the acre were used. In the side-dressing studies with minor elements, compounds supplying the respective minor elements were used at the following rates to the acre: magnesium sulfate, 80 pounds; manganese sulfate, 80 pounds; iron sulfate, 160 pounds; copper sulfate, 10 pounds; zinc sulfate, 2.5 pounds; borax, 1.25 and 2.5 pounds. These were applied in trenches near the row.

The 4–8–7 fertilizer applied on the two areas used for testing fertilizer mixtures of known composition, plus a minor element, was mixed from

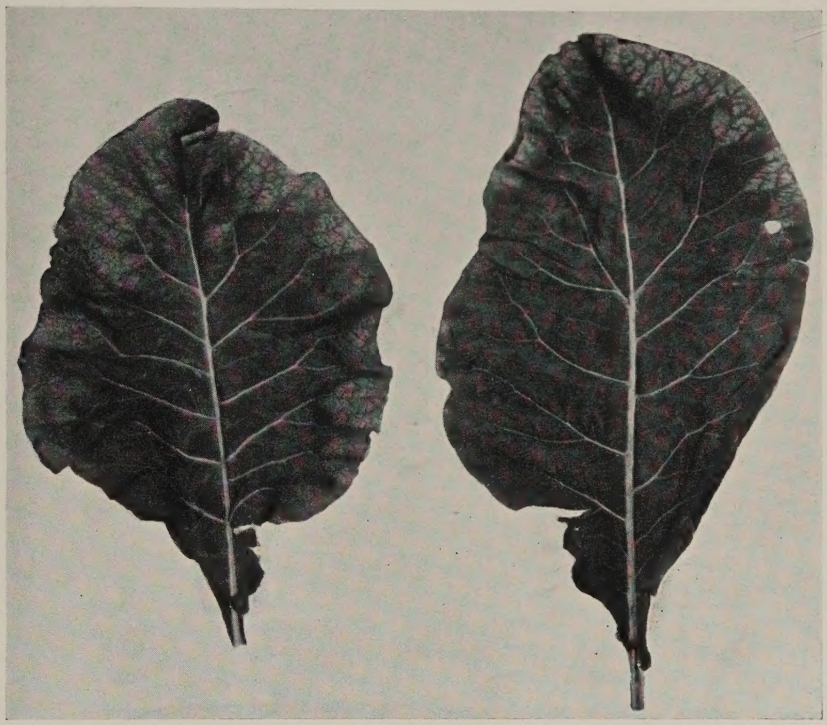


FIGURE 4. BORON-DEFICIENCY SYMPTOMS ON CAULIFLOWER LEAVES
At the edge of the leaf a distinct purple color develops, and an orange-yellow band extends inward from the periphery to a depth of ½ to 1½ inches

nitrate of soda 16 per cent N, sulfate of ammonia 20.8 per cent N, superphosphate 16 per cent P_2O_5, and muriate of potash 52 per cent K_2O. There were four lots of this fertilizer, each of which contained a compound supplying a minor element at the following rates to the acre: manganese sulfate, 250 pounds; iron sulfate, 250 pounds; borax, 5.0 pounds; borax, 2.5 pounds. The fertilizer was broadcast at the rate of 2 tons to the acre.

On the 1936, 1937, and 1938 experimental areas, hydrated lime, with a high percentage of calcium unless otherwise designated, was applied with a lime-spreader at the desired rate to the acre. The soil was harrowed immediately and thoroughly, to ensure even distribution of the lime. From 1 to 27 days prior to setting the plants, a 4–8–7 fertilizer was broadcast by hand on all of the plots at the rate of 2 tons to the acre (the rate generally used in the region). For the 1936 and 1937 experiments, borax was mixed with four lots of the 4–8–7 fertilizer so that 0, 5, 10, and 25 pounds of borax to the acre, respectively, was added to the soil when the fertilizer was broadcast at the rate of 2 tons to the acre. In the 1938 experiments the treatments were 0, 5, 10, 25, 5 + 5, and 5 + 25 pounds of borax to the acre, respectively. The treatment designated as "5 + 5" in experiments 5 and 6 (table 2, page 12) was identical with the 5-pound borax treatment until

TABLE 1. SUMMARY OF EXPERIMENTAL PROCEDURE

Year and experiment no.	Soil type	Time of plowing	Lime (tons to the acre)	Date of application of 2 tons to the acre of a 4–8–7 fertilizer	Variety of cauliflower and date of setting	Soil reaction during the experiment (pH)	Date of first and of last harvest	Date of first surface browning
1936 Exp. 1	Lackawanna silt loam	Spring, 1936	Spring, 1936 Hydrated lime 2.0	May 27, 1936	Super Snowball June 8, 1936	6.0–6.6	July 27, 1936 Sept. 29, 1936	June 29, 1936
1936 Exp. 2	Culvers silt loam	Fall, 1935	Fall, 1935 Hydrated lime 1.0 Spring, 1936 Hydrated lime 2.0	May 26, 1936	Super Snowball June 22, 1936	5.8–6.4	Aug. 11, 1936 Oct. 2, 1936	Aug. 11, 1936
1937 Exp. 3	Barbour silt loam	Spring, 1937	Spring, 1937 Hydrated lime 2.0	May 26, 1937	Super Snowball June 5, 1937	6.2–6.4	Aug. 4, 1937 Aug. 30, 1937	Aug. 4, 1937
1937 Exp. 4	Walton silt loam	Fall, 1936	Spring, 1937 Ground limestone 3.0	May 31, 1937	Super Snowball June 15, 1937	5.3–6.4	Aug. 6, 1937 Sept. 3, 1937	Aug. 10, 1937
1938 Exp. 5	Lackawanna silt loam	Fall, 1937	Spring, 1938 Hydrated lime 2.0, high MgO	June 7, 1938	Improved Holland Erfurt June 8, 1938	6.0–6.4	July 26, 1938 Sept. 6, 1938	Aug. 4, 1938
1938 Exp. 6	Walton silt loam	Spring, 1938	Spring, 1938 Hydrated lime 1.5, high MgO	May 28, 1938	Super Snowball June 2, 1938	6.6–7.0	July 26, 1938 Aug. 5, 1938	July 15, 1938
1938 Exp. 7	Walton silt loam	Fall, 1937	Spring, 1938 Hydrated lime 2.0	July 3, 1938	Improved Holland Erfurt July 8, 1938	6.4–6.7	Oct. 5, 1938 Oct. 24, 1938	No surface browning of head

the plants were approaching the heading stage, at which time borax at the rate of 5 pounds to the acre was broadcast over the plots marked "5 + 5" in experiments 5 and 6. The treatment designated as "5 + 25" in experiment 7 (table 2) received broadcast 25 pounds of borax to the acre after the heads had formed. These broadcast applications of borax were made because it was believed that cracking of the pith of the stem could be prevented if a supply of boron adequate for plant growth could be maintained in the soil throughout the life of the plant. All treatments in the 1936, 1937, and 1938 experiments were made in quintuplicate. The latin-square arrangement of treatments was used for the 1938 experiments.

Planting and care of the crop

The plants used were of either the Super Snowball or the Improved Holland Erfurt type, and were grown in coldframes by the growers. Plant-setting was done by machine and the skips were filled in by hand. Plant-spacing ranged from 12 to 18 inches in the row and from 30 to 40 inches between rows. The plants were given good cultivation and were dusted with rotenone dust to control the imported cabbage worm.

Harvesting

The heads were harvested in the usual manner and were graded into three grades — no. 1, no. 2, and culls — on the basis of appearance and size. A plant weighing less than 2 pounds was rated as a no. 2 or a cull. As the plants were graded, they were examined for symptoms of browning both on the head and in the stem. The grading standards were adopted from packs put up by the better growers in the area, except for the weight limitations. The data obtained from these experiments consisted of a record on each plot of the total stand of plants, the number of grade-1 and grade-2 plants and their fresh weight, and the number of plants showing browning and their fresh weight, together with a record of the culls and frequent notes on the condition of the plants in the various plots. In 1938, records were taken of the weight of the head as trimmed for market, in addition to the weight of the entire plant above ground.

GREENHOUSE EXPERIMENTS

Two experiments were conducted in soil in the greenhouse, one in 1935–36 and the other in 1937–38. The object of the first experiment was to check the results of the field work with borax under more nearly controlled conditions, and that of the second was to determine the effects of borax on the fresh weight and on the distribution of the dry weight in boron-deficient and in normal cauliflower plants. All of these experiments were conducted in a greenhouse maintained at 60° to 70° F. during the day, and at 50°, 55°, or 60° F. during the night.

The soil used was a Lackawanna silt loam obtained from two farms in Delaware County. The soils were taken to a depth of 5 to 6 inches at the end of the growing season. The lot for the 1935–36 experiment was from a field where surface browning of cauliflower heads had been severe in 1935, and the soil for the 1937–38 experiment was from a field where cauliflower-browning had caused a complete loss of the crop in 1936. Both

these soils had received applications of 2 tons of hydrated lime and 2 tons of a 4–8–7 fertilizer to the acre, before the crops were grown in the fields.

In order that the major elements might not be limiting factors, a 4–8–7 fertilizer was mixed with the potted soil at the rate of 1 ton to the acre calculated from the surface area of the pot. This fertilizer was made from Baker's C.P. salts in the same proportions as were used for the mixtures in the field work. Borax was added to the pots in the quantities desired, either as amorphous borax or as a solution in distilled water.

Seeds of the Super Snowball and Improved Holland Erfurt types of cauliflower were sown, and the seedlings were pricked out into flats of the Lackawanna silt loam. When the plants were of a suitable size, they were transplanted into similar soil in well-paraffined clay pots 6, 8, or 12 inches in diameter. One plant was set in each pot and the plants were grown to maturity.

Careful records of observations were made at frequent intervals, and at the close of some of the experiments the fresh weight of each plant was taken as were also numerous measurements of the plants. Where dry-weight determinations were made, the material was placed in a ventilated oven and held at 65° C. until a constant weight was obtained.

LABORATORY METHODS

In order to get information as to the effect of boron on the internal structure and composition of the cauliflower plant, histological and microchemical studies were conducted in the laboratory.

Histological technique

The paraffin method was used to obtain most of the sections for the permanent mounts, but a few sections were made from fresh tissues. The samples for both methods were taken from cauliflower plants in all phases of the work, and included all of the plant parts and tissues. Therefore it was necessary to use several different combinations of killing and fixing agents. For tissues of the stem, the leaf, and the head, chromo-acetic or chromo-acetic-formalin killing and fixing solution was used; for root tissues, chromo-acetic-formalin solution and Regand's solution of 3-per-cent potassium bichromate 4 parts and formalin 1 part were used according to the laboratory manual for microtechnique of the Department of Botany at Cornell University.

The fixed material was thoroughly washed and gradually dehydrated through the ethyl-alcohol and the normal butyl-alcohol series. The dehydrated tissue was infiltrated with paraffin M.P. 45° C., and finally infiltrated with and embedded in paraffin M.P. 52° C. The rotary microtome was used for sectioning the embedded tissue, and the sliding microtome for sectioning the fresh tissue.

Sections of the various parts of the plant were stained with stain combinations such as Delafield's haematoxylin and rosin, crystal violet and erythrosin, and safranin and light green. Some of these stains were also used individually. The stained slides were mounted in balsam.

Microchemical technique

The microchemical tests used in this study are those given in Sophia H. Eckerson's *Microchemistry* (unpublished manual of laboratory outlines).

In order that parts of the head, stem, leaves, and roots might be sectioned immediately after removal from the plants, a few representative plants from soil and nutrient-solution cultures were moved temporarily from the greenhouse to the laboratory. For sectioning in a sliding microtome, the various plant parts were supported with elder pith thoroughly saturated with distilled water.

EXPERIMENTAL RESULTS

FIELD EXPERIMENTS

Experiments were conducted in the field in Delaware County during the four years 1935 to 1938. Since the experiments in 1935 were preliminary in nature and differed markedly from those conducted in the other three years, they are discussed separately.

Experiments in 1935

At the time when the experiments of 1935 were planned, no evidence was available to indicate the cause of cauliflower browning but it was thought the condition might result from a deficiency of some minor element. In the spring of 1935, six of these elements — magnesium, manganese, iron, copper, zinc, and boron — were applied in twenty-two fields in Delaware County, as described on page 6.

In addition to the experiments in the twenty-two fields mentioned above, two experiments were conducted on a larger scale. One of the areas selected for these experiments was a block of Culvers silt loam near a field that had produced cauliflower showing a considerable amount of browning in 1934; the other area was on Barbour silt loam which had been in timothy in 1934.

Plants in all of the experiments were examined at frequent intervals during the growing season, in order to determine when internal browning developed and the effect of the treatments on browning. The plants were split longitudinally through the stem, and notes were taken on the incidence of browning.

Browning was first observed on July 26 in the pith of Super Snowball plants growing on a well-limed and well-fertilized Lackawanna soil adjacent to one of the minor-element experiments. The lower leaves of a few plants that were heading prematurely showed the purple edges and orange-colored margins characteristic of boron-deficient cauliflower plants. Occasionally a stem was found to be hollow even before the head was visible. On August 8 it was observed that the plants growing on the broadcast treatments of borax had less water-soaked tissue around the cracks in the stems than was the case with plants having any other treatment. At this time of the season, internal browning and leaf symptoms were common well in advance of the surface browning. By August 15 nearly all of the plants except those treated with borax showed internal browning. Browning of the surface of the cauliflower heads also was appearing in several fields at this time. The effect of the borax treatments in preventing surface browning became increasingly significant as the plants approached maturity.

At the close of the season, in each of the twenty-two fields of minor-element experiments the borax treatment was the only one on which plants

developed free from internal and surface browning. In 45 per cent of these fields, internal browning was severe in the rows on both sides of the borax treatment. In several of the fields the surface of nearly every cauliflower head became brown except where borax had been used. The broadcast applications of borax prevented surface browning on both the Culvers and the Barbour soil types; however, there were occasional plants which showed minute water-soaked areas on the plants grown on the Barbour soil where 2.5 pounds of borax to the acre had been applied broadcast.

The elements magnesium, manganese, iron, copper, and zinc, when applied either in a trench or broadcast with the fertilizer, had no apparent effect on the prevalence or on the severity of the discoloration of the cauliflower head.

Experiments in 1936, 1937, and 1938

The results of the field studies in 1935, together with those of a greenhouse experiment reported subsequently in this bulletin, indicated that borax prevented cauliflower-browning. The results of seven experiments on four major soil types in 1936, 1937, and 1938, show that, where borax was added with the fertilizer, surface browning of cauliflower heads was satisfactorily prevented. Browning occurred on the no-borax treatments in five of the seven experiments. The severity of browning is indicated by the data in table 2. No browning developed on the plants grown in the borax-treated plots except in experiment 1, in which 1.3 and 0.5 per cent of the heads, respectively, showed browning where the applications of borax were 5 and 10 pounds to the acre.

TABLE 2. EFFECT OF BORAX ON THE INCIDENCE OF CAULIFLOWER-BROWNING IN FIELD EXPERIMENTS OF 1936, 1937, AND 1938

Borax (pounds to the acre)	1936		1937		1938		
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7
	Per cent of plants showing surface browning						
0	17.0	42.5	50.0	47.9	18.5	0.0	0.0
5	1.3	0.0	0.0	0.0	0.0	0.0	0.0
10	0.5	0.0	0.0	0.0	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5+5	....	....	....	....	0.0	0.0	....
5+25	....	....	....	....	....	....	0.0

In experiment 7, internal browning was severe in the stems of the plants on the no-borax plots but no surface browning was observed on any plants in the experiment. Evidently the boron deficiency was not acute enough to cause surface browning. Transverse cracking in the pith of the main stem was very common on the no-borax plots; much less cracking was observed in stems of plants grown on plots treated with 10 and 25 pounds of borax to the acre. The broadcast applications of borax were applied too late to have any retarding effect on the transverse cracking. In the Erfurt and late Snowball types, which produce heavy heads, transverse

cracking and hollow stem are always more severe than in the lighter strains of the Super Snowball type.

GREENHOUSE EXPERIMENTS

The soil studies with cauliflower in the greenhouse consisted of two experiments, which differed in plan and in purpose and for this reason are presented separately.

Experiment 1

The first experiment was conducted to check the results obtained in preliminary experiments made in the field in 1935. The soil used in this experiment was from a field of Lackawanna silt loam on which browning was severe in the summer of 1935.

In the fall of 1935, sixty 12-inch clay pots, well paraffined, were filled with soil, a uniform weight of soil being placed in the respective pots. A given amount of this soil, representing a depth of about 4 inches, was removed from each pot, and a 4–8–7 fertilizer containing the desired quantity of borax was mixed thoroughly with the soil. The fertilizer was applied at the rate of 1 ton to the acre. The six treatments were as follows: no borax, and 2.5, 5.0, 7.5, 15.0, and 25.0 pounds of borax, respectively, to the acre.

Sixty cauliflower plants of the Super Snowball strain were set on December 31, 1935, with ten plants in each of the six treatments. Each pot received an application of nitrate of soda C.P., at the rate of 50 pounds to the acre, as the plants were approaching maturity. Harvesting extended from March 10 to April 4, 1936, and during that time all the plants were cut at market maturity.

The effect of borax on plant growth is shown in table 3. The heads of all the plants grown in soil to which no borax was added, showed severe browning and were very bitter in flavor. Transverse cracks were common on the stems of several plants in this treatment. Symptoms of boron deficiency on the leaves were common on all of the plants in the no-borax plots, and four heads showed slight internal browning where 2.5 pounds of borax to the acre was used. On the 5.0-pound borax treatment, two plants showed minute spots in the interior of the apical part of the main stem. No surface browning occurred on any of the borax-treated plants, and no in-

TABLE 3. EFFECT OF BORAX ON GROWTH OF CAULIFLOWER PLANTS IN 12-INCH POTS IN 1935–36

Borax (pounds to the acre)	Average fresh weight per plant (grams)			Average number of leaves per plant
	Total plant exclusive of roots	Head	Three root systems	
0.0	641.4	194.4	114	24.3
2.5	700.6	278.7	...	22.6
5.0	747.6	292.8	...	23.4
7.5	784.2	280.8	153*	26.0
15.0	785.3	302.0	171	24.8
25.0	844.9	303.3	195	25.9
Least difference at odds of 19:1	72.3	47.5	....	....

*Observations from one plant.

ternal symptoms developed on plants supplied with 7.5, 15.0, or 25.0 pounds of borax to the acre. The effect of borax on the incidence of browning is shown in figure 5.

FIGURE 5. EFFECT OF BORAX ON THE INCIDENCE OF BROWNING OF CAULIFLOWER GROWN IN 12-INCH POTS IN THE GREENHOUSE EXPERIMENT OF 1935

The head on the left, showing surface browning, was grown in Lackawanna silt loam deficient in boron; the normal head on the right was grown in similar soil supplied with 25 pounds of borax to the acre

The data on fresh weight of the entire plant aboveground showed that borax applied at the rate of 5.0, 7.5, 15.0, and 25.0 pounds to the acre increased the yield significantly over that with the no-borax treatment. While the difference in weight of plants between any two consecutive treatments was not statistically significant, the plants grown on the 25.0-pound borax treatment were significantly larger than those grown on the 5.0-pound treatment. Similarly, 7.5 pounds of borax to the acre produced significantly larger plants than did the 2.5-pound treatment. The differences in weight of plants as between the 7.5 pound, the 15.0-pound, and the 25.0-pound treatment were not significant.

The weight of the head free of leaves was significantly less on the no-borax treatment than on any of the borax treatments. There were no significant differences in weight of heads as among the various borax treatments.

Although the root systems of only ten plants out of forty were washed free of soil and their weight recorded, it is evident that borax increased root development as much as it did the development of the top, or even more. The root system was so large in the 12-inch pots of the 25-pound borax treatment (figure 6) that the entire soil mass was lifted intact from some of the pots; while the root system in the plants grown without borax was so small and brittle that only the surface 2 or 3 inches of soil was lifted from the pot.

The number of leaves per plant varied only slightly among the six treat-

FIGURE 6. EFFECT OF BORAX ON GROWTH OF THE ROOT SYSTEM OF CAULIFLOWER PLANTS
The two root systems on the left are from plants grown in the greenhouse in Lackawanna silt loam to which borax was added at the rate of 25 pounds to the acre; the two on the right are from plants grown in the same type of soil without borax

ments. This was to be expected, since the browning was not severe until after most of the leaves had been formed.

Experiment 2

Although cauliflower-browning was completely prevented where sufficient amounts of borax were supplied, the way in which boron prevents browning is not understood. A second greenhouse experiment was conducted in 1937–38, in an attempt to obtain some information on the possible role of boron in the development of the cauliflower plant.

The plan of this experiment was to obtain fresh- and dry-weight data from cauliflower plants grown without borax and with 10 pounds of borax to the acre. The procedure differed from the 1935–36 experiment in that the plants were grown in 6-inch pots. Eighty-four plants of the Super Snowball strain of cauliflower were transplanted, one plant to a pot, on November 23, 1937. Half of the plants received 10 pounds of borax to the acre, and half received no borax. On January 9 and January 23, 1938, all the pots were supplied uniformly with sodium nitrate at the rate of 50 pounds to the acre. On the latter date, heads were forming on the plants

grown in the soil to which borax had been added. Symptoms of browning were common on the no-borax series at this time, but heads did not form until February 4. The severity of the boron deficiency is illustrated in figure 7. On February 27, fifteen uniform plants in each treatment were cut and their weights were recorded as shown in table 4. The remaining plants were saved for laboratory studies.

FIGURE 7. SEVERE SURFACE BROWNING OF CAULIFLOWER PLANTS GROWN IN BORON-DEFICIENT SOIL IN THE GREENHOUSE IN 1937–38

The data in table 4 show that where 10 pounds of borax to the acre was supplied, the fresh weight both of the entire plant exclusive of roots, and of the head, was greater by significant odds than was the case where borax

TABLE 4. EFFECT OF BORAX ON THE FRESH WEIGHT AND THE DRY WEIGHT OF BORON-DEFICIENT AND OF NORMAL CAULIFLOWER PLANTS

Number of plants	Borax (pounds to the acre)	Average fresh weight per plant (grams)		Average dry weight per plant (grams)		Dry weight of head as per cent of fresh weight
		Entire plant aboveground	Head	Entire plant aboveground	Head	
15	0	88.5	24.0	11.18	2.414	10.06
15	10	103.6	43.0	10.94	3.726	8.67
Odds	.....	>4999:1	>4999:1	1:1	>4999:1	>4999:1

was omitted. The fresh weight of the head in the treated plants was about 44 per cent greater than that in the boron-deficient plants. These differences were easily visible before the plants were cut.

When the dry weight of the heads is expressed as per cent of the fresh weight of the heads, it is seen that the head in the boron-deficient plants contained a higher percentage of dry matter (10.06 per cent) than that contained in the head in the normal plants (8.67 per cent). This significant relationship indicates that an abundant supply of carbohydrates was translocated to the head. These findings in this experiment are in line with the results obtained by Eltinge (1936), in which it is pointed out that the dry weight as per cent of the fresh weight in *Zea mays* is higher in boron-deficient plants than in normal plants.

There was no significant difference in the actual amount of dry matter contained in the aboveground parts of the plants of the two treatments, even though their fresh weights differed by 14.5 per cent.

The heads of the borax-treated plants actually contained more dry matter than did the heads of the plants from which borax was omitted; however, the percentage dry weight of the head was higher in the plants grown without borax.

HISTOLOGICAL STUDIES

The histological investigation was undertaken to determine how boron deficiency is manifested in the tissues of various parts of the cauliflower plant.

The vascular tissue of cauliflower is arranged in the stem in a circle of distinct vascular bundles surrounding a relatively large continuous area of pith. The parenchyma cells of the pith and the cortex are the first to show evidence of boron deficiency. In the pith of the stem, scattered cells enlarge and partly close the adjacent intercellular spaces.

The first manifestation of boron deficiency as related to individual pith cells was observed in the intercellular spaces. A translucent mucilaginous, or gummy, material is formed here, which, as it fills the intercellular spaces, gives the tissue a water-soaked appearance. This intercellular material passes from the almost colorless state through shades of amber and orange and finally becomes deep brown, in this last stage taking a positive stain for

lignin. In the early stages only an occasional intercellular area turns brown, as is shown in figure 8. It is seen that the affected cells are very large.

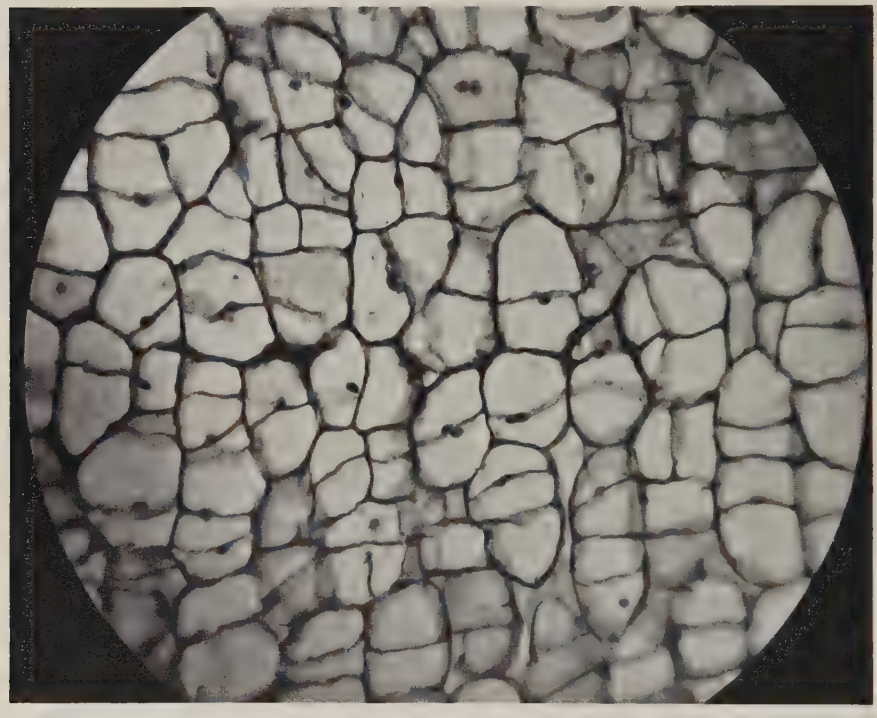


FIGURE 8. LONGITUDINAL SECTION OF A CAULIFLOWER STEM, SHOWING EARLY STAGES OF INTERNAL BROWNING BETWEEN THE PITH CELLS. × 430

The deeply stained intercellular areas in the center and to the left of the center show where the mucilaginous material is first observed

Many cauliflower plants grown in the field and in the greenhouse developed cracks in the pith. These cracks, mentioned earlier in the description of cauliflower-browning, are a result of boron deficiency in the plant. The histological studies show that gumming of the intercellular spaces nearly always starts near the center of the stem or the center of the branches of the head, and spreads toward the periphery of the pith. As the stem or branch elongates, the pith is pulled apart along the suture formed by the gumming of the intercellular spaces and cell walls. This condition is illustrated in figure 9.

In the parenchymatous tissue of the head, the cells enlarge and their walls exhibit the same injury as do the walls of cells in the pith of the stem. The cellular disturbance is shown in figure 10, together with the destruction of the meristematic cells of one of the branches.

In the petiole some of the parenchyma cells enlarge, causing blisters to form along the upper side of the midrib. Within these blisters the

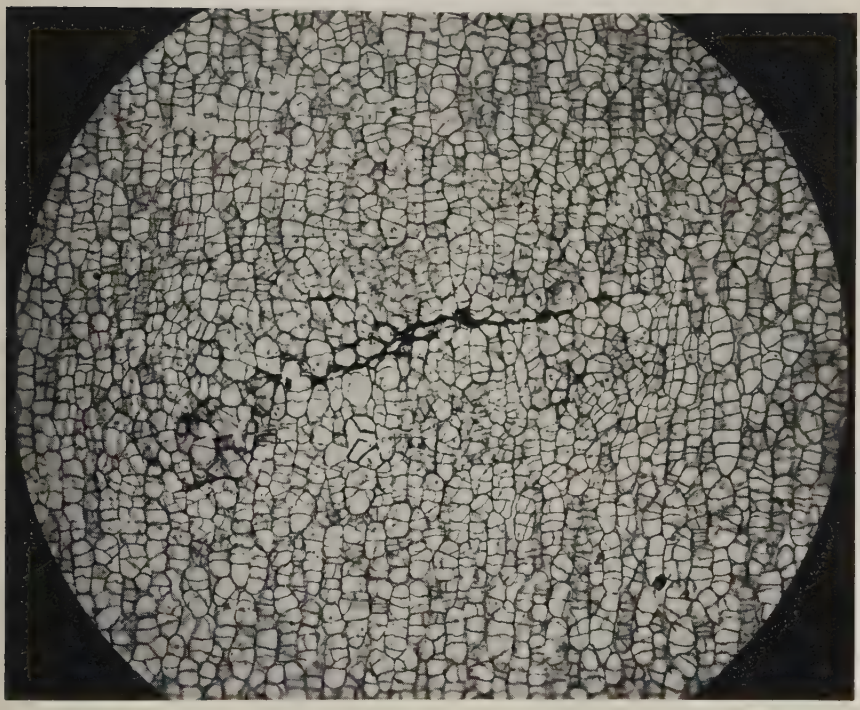


FIGURE 9. TRANSVERSE SUTURE IN THE PITH OF A STEM, FORMED BY THE GUMMING OF INTERCELLULAR SPACES AND CELL WALLS. × 100

As the stem elongates, the tissue in this region pulls apart and transverse cracks in the pith of the stem are visible

disorganization of the tissue is similar to the breakdown of the pith cells in the stem.

In the vascular tissue the amber-colored substance is fairly generally distributed, but it is seldom found in the older vascular tissue of the stem even though the pith cells in this region are disorganized. The manner in which the browning is distributed in the vascular bundles is shown in figure 11. The filling of the intercellular spaces is at first most clearly visible between the walls of the phloem cells. Occasionally an entire vascular bundle is affected, but the number of bundles plugged in any given section seems too small to account for death of the surrounding tissue because of inadequate channels of transport.

In the terminal regions of the main stem or of the branches of the curd, where the phloem and the xylem have not been differentiated, the intercellular accumulations appear in the meristem and the procambium. The accumulations between the cell walls are shown in figure 12. Proceeding toward the apex of the stem, the intercellular accumulations envelop the meristematic cells of the branches of the head so completely that in the early stages of severe boron deficiency these cells appear to be water-soaked.

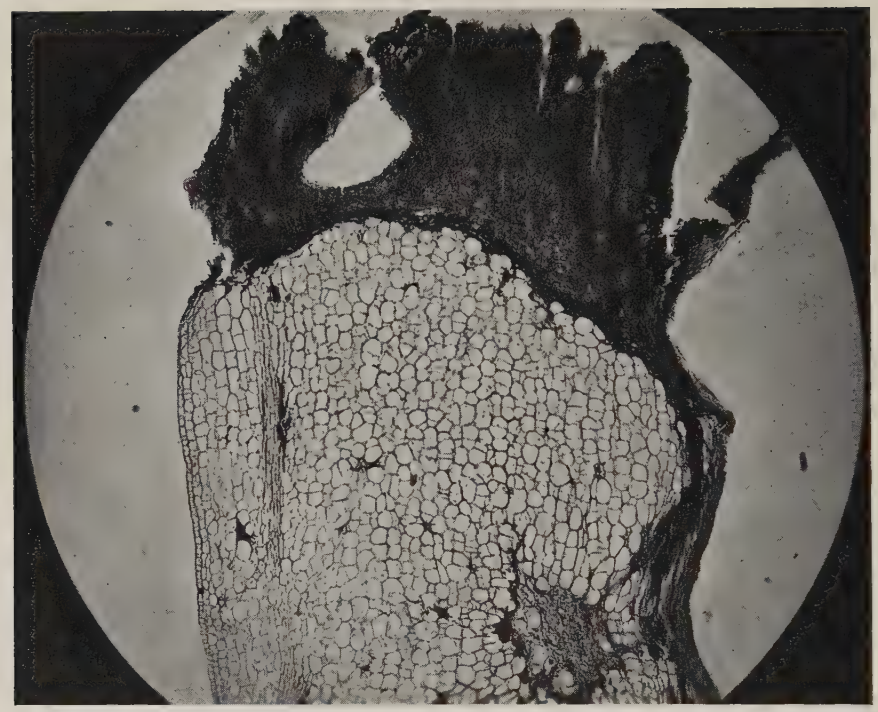


FIGURE 10. LONGITUDINAL SECTION OF A BRANCH OF A CAULIFLOWER HEAD, SHOWING DESTRUCTION OF CELLS OF THE MERISTEMATIC REGION AND OF THE PITH, THE CORTEX, AND THE VASCULAR SYSTEM AS A RESULT OF BORON DEFICIENCY. × 100

The main vascular bundles in the petiole are partly surrounded by the amber-colored substance. Usually the breakdown is most severe in the phloem, with large aggregates of the gummy substance separating the phloem cells. When the tissues have suffered from a lack of boron for several days, disruption of these enlarged phloem cells is not uncommon. The cells in the area of the conducting tissue which usually weaken first, are located between the larger vascular bundles. In the tissues of a normal petiole the cell walls are distinct and are free from the gummy substance.

Symptoms of boron deficiency in the root are similar in some respects to those observed in the aboveground part of the plant. The rootcap in the boron-deficient plant has about the same volume as has the rootcap in the normal root, but the cells of the former appear somewhat larger. The outer walls of the rootcap cells at the growing point in the boron-deficient plants appeared to be covered with the amber-colored substance. Some of the cells at the growing point also showed the intercellular accumulation, as well as disorganization. The protoplasm of the rootcap cells appeared normal except for the presence of several very large vacuoles in a few of the cells. Numerous secondary-root primordia were noted abnormally close to the growing point in the boron-deficient roots. Warington (1926),

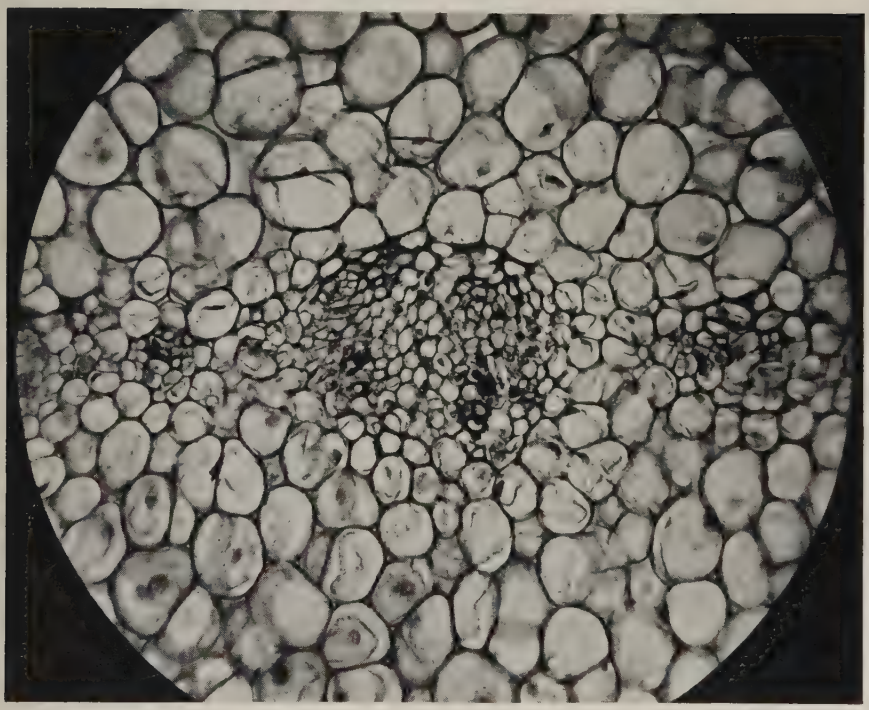


FIGURE 11. CROSS SECTION OF A VASCULAR BUNDLE IN A BORON-DEFICIENT BRANCH OF A CAULIFLOWER HEAD. × 430

The brown substance has accumulated in the intercellular spaces of the phloem and the phloem parenchyma (top) and in similar areas of the xylem parenchyma (bottom)

and Sommer and Sorokin (1928), have discussed these abnormalities of secondary-root primordia.

A typical cross-section of a boron-deficient root shows that in the region of cell elongation some of the areas between the cortical parenchyma cells are filled with the amber-colored substance. One characteristic feature of the boron-deficient root is that the endodermis and tissues of the stele are normal.

In the boron-deficient root the amber-colored material is most abundant in the intercellular spaces between the first and the second ring of cortical parenchyma cells. These areas actually separate the cells and extend for some distance between them, as is shown in figure 13. It is seen that the cell walls are intact along the region of intercellular accumulation.

Another peculiarity of the boron-deficient root is the presence of a strip of lightly stained substance, resembling cytoplasm, within the cortical parenchyma cells lying adjacent to the endodermis. This band within each cell extends circumferentially in a ring from cell to cell and longitudinally in a sheet, thus forming a cylinder surrounding the endodermis. A cross section of this cylinder is shown in figure 14. From the band within the cells many small strands radiate to the periphery of the respective cells,

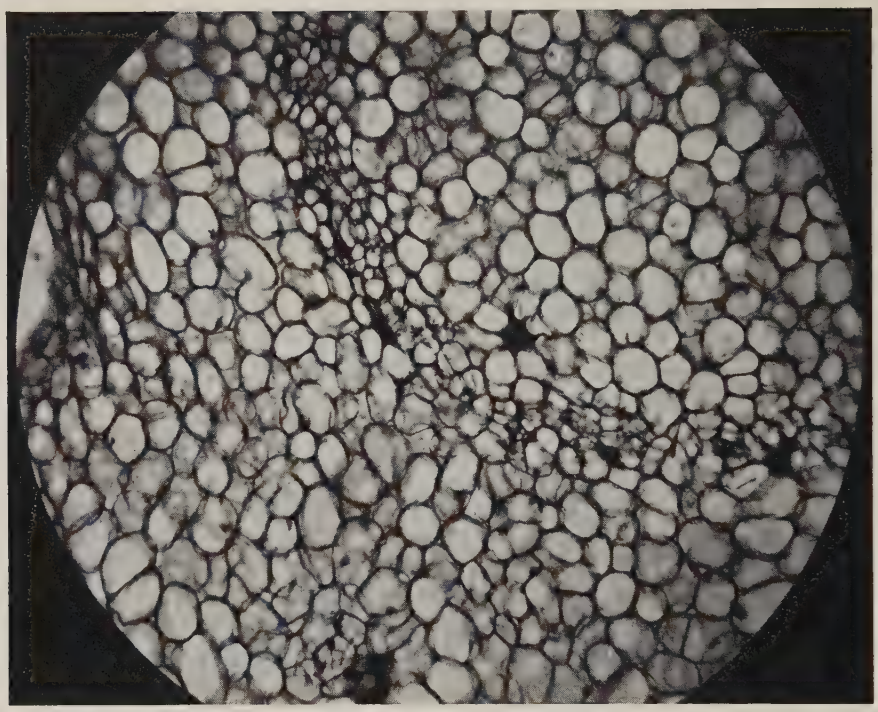


FIGURE 12. CROSS SECTION OF A BRANCH OF A BORON-DEFICIENT CAULIFLOWER HEAD IN THE REGION OF UNDIFFERENTIATED VASCULAR BUNDLES. × 430

The band of procambial cells appears normal, but the cortical parenchyma cells at the left of this band and a group of pith cells at the right of the band are affected

where they branch to form a network which can be seen in either a longitudinal or a tangential section (figures 15 and 16). The tangential section (figure 16) shows that the intracellular reticulation is continuous for some distance around the stele. A normal-appearing nucleus was observed in one of the reticulated cells. The band and the radiating strands were stained with crystal violet, and they were observed only in sections of boron-deficient plants where vascular tissue was well developed.

MICROCHEMICAL STUDIES

In this investigation an attempt was made to determine the origin and nature of the intercellular material, and to determine whether there was any difference in the content of reducing sugar, in boron-deficient and in normal cauliflower plants. The tissues of the cauliflower head are particularly adapted to microchemical studies because they are devoid of plant pigments and are solid enough to facilitate fresh sectioning. Sections of tissues of the head showing the first symptoms of internal browning were examined under a low-power lens. With the aid of glass rods drawn out to a fine point, the intercellular substance was probed. This material appeared somewhat sticky as it was drawn out with the needle; but it retracted after the needle was removed, as if it were highly viscous.

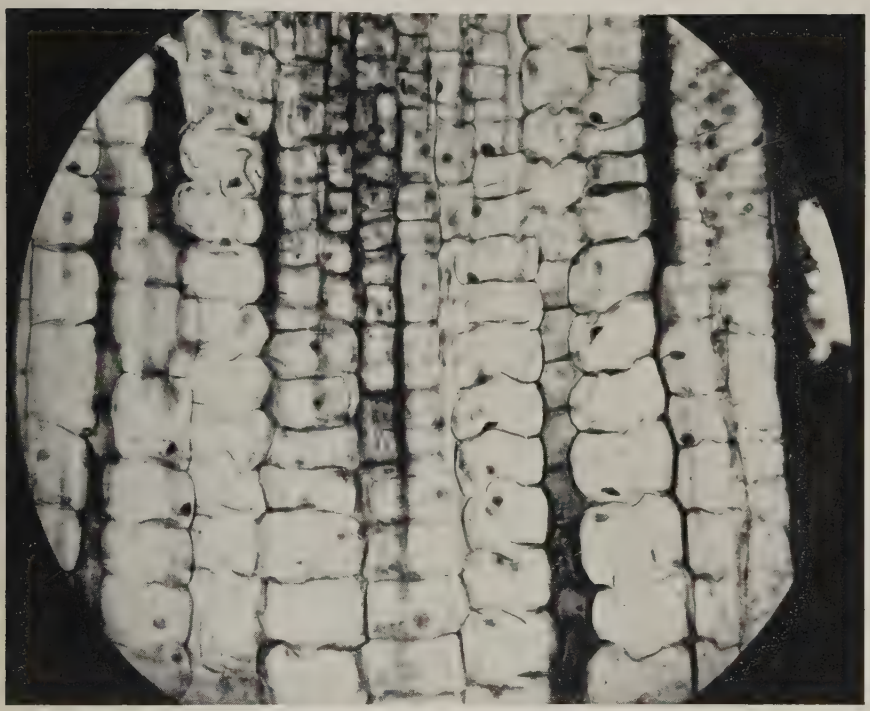


FIGURE 13. LONGITUDINAL SECTION OF A BORON-DEFICIENT CAULIFLOWER ROOT. × 430
The dark strips separating the cortical parenchyma cells are filled with the amber-colored substance

The middle lamella was examined next, because the disturbance appeared to be in that area. Some sections were stained with ruthenium red and others were stained with methylene blue, to see whether the middle lamella had been altered. Ruthenium red gave a negative test for pectic substances in the region of intercellular accumulation. It is possible that the middle lamella was masked by the intercellular substance so that a positive test was not visible; on the other hand, a change in the composition of pectic materials of the middle lamella may have accounted for the negative test. Methylene blue gave a positive test for pectic substances in primordial tissue of a head showing slight surface browning.

Cellulose tests, using iodine–potassium-iodide and sulfuric acid, were made on cross sections of a petiole in the region of a blister. A blue color, indicating the presence of cellulose, appeared throughout the cell walls in the entire area. In the region of the blister the walls of the parenchymatous cells appeared much thicker, and retained the blue color nearly twice as long as in the normal tissue. In affected tissue the suture and the adjacent cell walls were so dark at the start that the cellulose test was faint. When such a section was treated with concentrated H_2SO_4, the gummy substance dissolved or the color disappeared in about forty minutes.

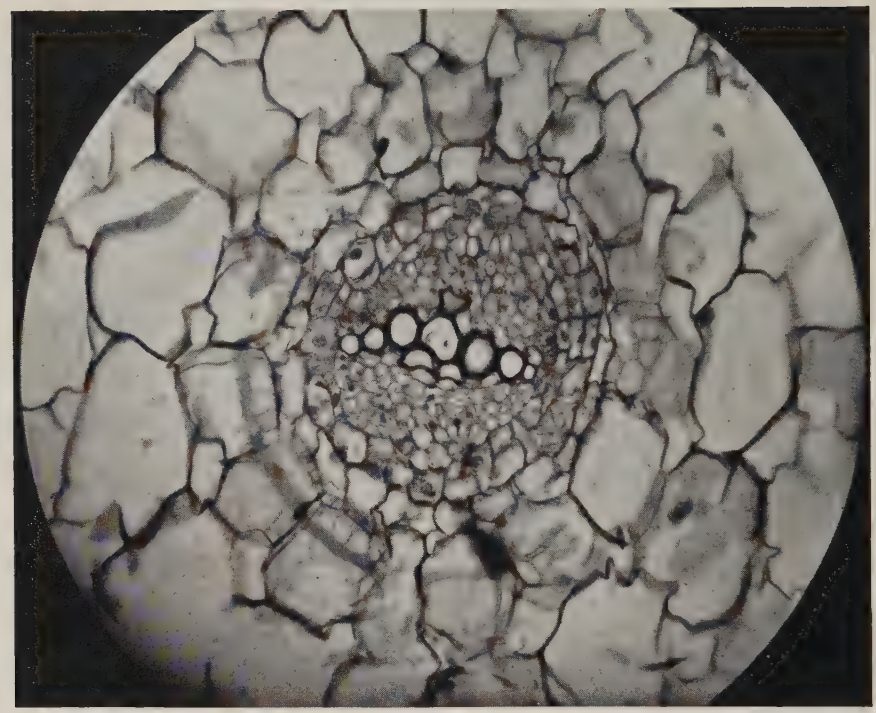


FIGURE 14. CROSS SECTION OF A BORON-DEFICIENT CAULIFLOWER ROOT. × 430
The bands of cytoplasm within the cortical parenchyma cells lying adjacent to the endodermis form a cylinder around the stele

During the course of this work, many sections from root, stem, head, and leaves showing browning were treated with phloroglucin and hydrochloric acid. In every case the central part of the gummy orange-colored substance turned violet immediately, which is a positive test for lignin. In most cases there was a gradation in color from cherry red to violet as the reaction proceeded from the margin to the center of the intercellular accumulations. This indicates that the chemical nature of the material differs throughout its mass, and that it was in a transitory stage when treated. The phloroglucin–hydrochloric-acid test indicates that the final product possesses some of the properties of lignin. This assumption is supported by the fact that the gummy brown material dissolves readily in 50-per-cent chromic acid. If pentoses are present in tissue treated with phloroglucin and hydrochloric acid, gentle heating will bring out a cherry-red color. In these tests no change was observed after gentle heating of the slide and section.

Although no definite injury was noted within the primordial cells of the branches of the curd, several tests for proteins were made. A few sections were treated for the biuret group ($CONH_2$), but in all cases the tests were negative. The presence of tyrosine in these brown areas was detected with Millon's reagent and also by the xanthoproteic reaction. The early stages

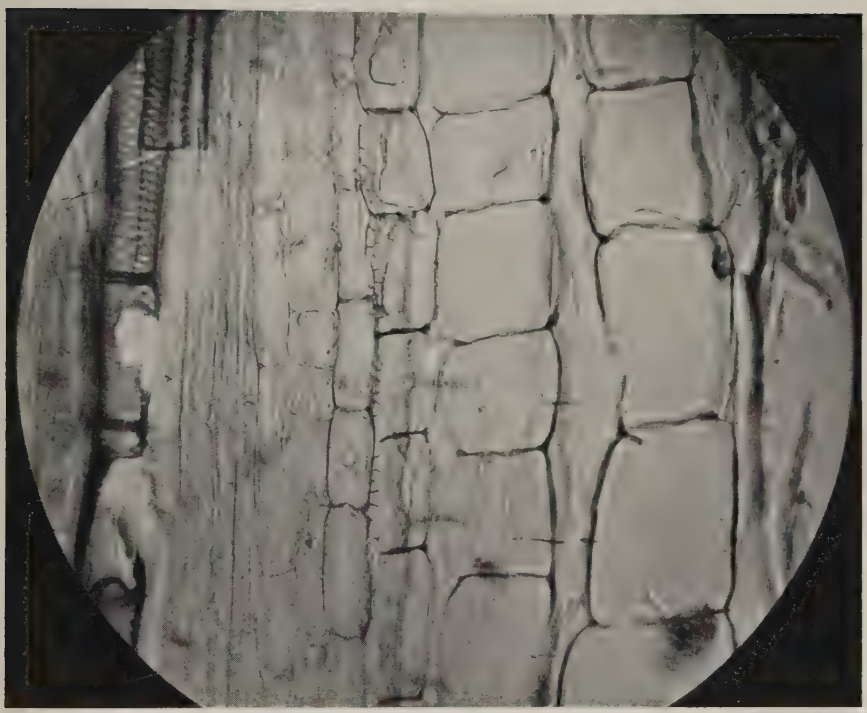


FIGURE 15. LONGITUDINAL SECTION OF A BORON-DEFICIENT CAULIFLOWER ROOT. × 430
The sheet of cytoplasm, with the strands radiating to the walls of the cortical parenchyma cells lying adjacent to the endodermal cells, is shown running vertically through the row of cells to the left of the center

of the intercellular accumulation were not accompanied by a positive test for tyrosine, however. Since the tyrosine is associated with protein decomposition, one would expect to find a positive test in some of the cells in the early stages of boron deficiency if the protoplasm were affected; but no positive test was observed.

Many sections from the branches of the head and from the roots were treated on slides with copper tartrate and a drop of 15-per-cent NaOH, for the presence of reducing sugars. Glucose was found in all of the treated sections, even within the cells that were in contact with the intercellular substance. In the sections from boron-deficient plants there were at least twice as many Cu_2O crystals as in the tissues of the normal plant. This is interpreted as meaning that reducing sugars are abundant rather than deficient in boron-starved tissue. The results obtained with some cross sections of boron-deficient roots indicated that a great deal of the copper was reduced outside of the cell walls. Normal root tissue did not reduce copper along the cell walls in this manner.

DISCUSSION AND SUMMARY

A survey made in the fall of 1934 of approximately 150 farms in the Catskill Mountain area, covering 443 acres of cauliflower, showed that

FIGURE 16. TANGENTIAL SECTION OF A BORON-DEFICIENT CAULIFLOWER ROOT. × 430
The cortical parenchyma cells showing the network of strands form a sheath around the stele

about 30 per cent of the crop was unfit for market in that year because of browning of the heads. It was hoped that the results of the survey would give a clue as to the cause of the browning, but no clue was found and it was decided to conduct a series of field experiments. These were started in 1935 in cooperation with the Delaware County Farm Bureau, and were continued through the growing seasons of 1936, 1937, 1938, and 1939.

The primary objects of the studies reported here were to determine the causes of cauliflower-browning and to find a remedy if possible. The investigation consisted of field experiments in Delaware County, greenhouse experiments with soil from affected fields and with culture solutions, and histological and microchemical studies.

When cauliflower-browning is severe, the surface of several or of all of the branches of the head appears water-soaked. These water-soaked areas soon turn rusty-brown, and in periods of dry weather the brown surfaces become hard and woody. Preceding this surface discoloration, there are small spherical areas, water-soaked in appearance, scattered through the pith of the main stem and the branches of the head. The affected head not

only is unsightly, but is bitter in flavor both in the raw and in the cooked state, even when no surface browning is evident.

Experiments of a preliminary nature were conducted with six minor elements in twenty-two fields during the growing season of 1935. The results of these studies showed that where borax had been mixed with the fertilizer and broadcast so as to supply 5 pounds of borax to the acre, or where borax had been applied at the rates of 1.25 and 2.5 pounds to the acre in trenches near the row, surface browning was satisfactorily prevented.

Data obtained from field experiments in 1936, 1937, and 1938 in Delaware County, in which borax was used at the rates of 0, 5, 10, 25, and 30 pounds to the acre, showed that browning of cauliflower heads was satisfactorily prevented by applications of 5 pounds of borax to the acre, and was completely prevented when 10 or more pounds of borax to the acre was applied.

The results of the field work are substantiated by two experiments conducted in the greenhouse with Lackawanna silt loam from cauliflower fields affected with browning. In the 1935–36 greenhouse experiment, records made on plants grown without borax, and with 2.5, 5.0, 7.5, 15.0, and 25.0 pounds of borax, respectively, to the acre, showed that surface browning was completely prevented on all plants where borax was added to the soil, and that no internal browning occurred on plants supplied with 7.5, 15.0, or 25.0 pounds of borax to the acre. All of the boron-deficiency symptoms developing in the greenhouse studies were identical with those observed in the field experiments.

While the yield data for the weight of the entire plant aboveground showed that the difference between any two consecutive treatments was not statistically significant, they showed that the plants on the 5.0-pound borax treatment were significantly larger than those grown without borax and that the plants grown on the 25.0-pound treatment were significantly larger than those grown on the 5.0-pound treatment. Similarly, there was a significant difference in weight as between the plants grown on the 2.5-pound treatment and those grown on the 7.5-pound treatment, in favor of the higher rate of borax application. The differences as between the 7.5-pound, the 15.0-pound, and the 25.0-pound treatment were not significant.

The yield data for weight of the head showed that cauliflower plants supplied with borax, at any rate used, produced heads significantly larger than did plants grown without borax. The differences in weight of heads as among the five treatments with borax were not significant.

In a second experiment in the greenhouse, in which plants were grown without borax and with 10 pounds of borax to the acre, the previous results were confirmed. The results on the dry-weight determinations for the two lots of fifteen plants each, in this experiment, are of particular interest, since they showed that the plants on the two treatments contained nearly the same amount of dry matter even though their fresh weights differed by 14.5 per cent in favor of the borax treatment. In this case the fresh weight of the head in the treated plants was increased by about 44 per cent over that in the boron-deficient plants.

When the dry weight of the heads is expressed as per cent of the fresh weight of the heads, it is seen that there was a higher percentage of dry matter in the head in boron-deficient plants than in normal plants, indicating that an abundant supply of carbohydrates was translocated to the head.

There are differences of opinion as to where boron deficiency is first manifested in green plants. In the present study, the first symptoms of boron deficiency were found in the parenchyma cells of the pith and the cortex of the stem. These results are in fair agreement with the findings of Wadleigh and Shive (1939), who state that, in cotton, scattered cells of the pith and the cortex are first to manifest boron starvation. The findings are further supported by the work of Martin (1934) on sugar cane, of Eltinge (1936) on *Zea mays*, and of Dennis and O'Brien (1937) on swedes.

The results obtained by Warington (1926), Haas and Klotz (1931), Rowe (1936), and others, indicate that boron deficiency is first manifested in the conducting system of plants. If the conducting system is congested with products of photosynthesis, and the tissue dies from a lack of energy-supplying material in the region of meristematic activity, one would expect that the boron-deficient roots would be very low in sugars. Microchemical tests for reducing sugars in the root and in the head of both boron-deficient and normal plants showed that in all cases the amounts of copper reduced in the boron-deficient roots and heads were far in excess of those reduced in the tissues of normal plants. There is little reason to doubt that, in advanced stages of boron deficiency, disorganization within the conducting system has occurred; but the fact that sugars are abundant when the intercellular spaces are being filled with the translucent mucilaginous substance, is evidence that the supply of energy materials is not exhausted in these areas. Wadleigh and Shive (1939) have found that the concentration of sugars is abnormally high in the pith cells of boron-deficient cotton plants. Johnston and Dore's (1929) data on the analysis of the tomato plant showed that the percentage of sugar, both reducing and total, in roots of boron-deficient plants was about double that found in roots of normal plants, although no emphasis was placed on this fact.

REFERENCES

DENNIS, R. W. G., AND O'BRIEN, D. G. Boron in agriculture. West of Scotland Agr. Coll. Research bul. 5: 1–98. 1937.

ELTINGE, ETHEL TABER. Effect of boron deficiency upon the structure of *Zea mays*. Plant physiol. **11**: 765–778. 1936.

HAAS, A. R. C., AND KLOTZ, L. J. Some anatomical and physiological changes in citrus produced by boron deficiency. Hilgardia **5** (1930–31): 175–196. 1931.

JOHNSTON, EARL S., AND DORE, W. H. The influence of boron on the chemical composition and growth of the tomato plant. Plant physiol. **4**: 31–62. 1929.

MARTIN, J. P. Boron deficiency symptoms in sugar cane. Hawaiian planters' rec. **38**: 95–107. (*Abstr. in* Bibliography of references to the literature on the minor elements and their relation to plant and animal

nutrition [originally compiled by L. G. Willis], 3d ed., p. 94. 1939.) 1934.

ROWE, ETHEL A. A study of heart-rot of young sugar-beet plants grown in culture solutions. Ann. bot. [London] **50**: 735–746. 1936.

SOMMER, A. L., AND SOROKIN, HELEN. Effects of the absence of boron and of some other essential elements on the cell and tissue structure of the root tips of *Pisum sativum*. Plant physiol. **3**: 237–260. 1928.

WADLEIGH, CECIL H., AND SHIVE, JOHN W. A microchemical study of the effects of boron deficiency in cotton seedlings. Soil sci. **47**: 33–36. 1939.

WARINGTON, KATHERINE. The changes induced in the anatomical structure of *Vicia Faba* by the absence of boron from the nutrient solution. Ann. bot. [London] **40**: 27–42. 1926.